Mismatch Repair in Extracts of Werner Syndrome Cell Lines

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ABSTRACT

Werner syndrome (WS) is an autosomal recessive disease, the phenotype of which is a caricature of premature aging. WS cells and cell lines display several types of genetic instability, and WS patients have an increased risk of developing cancer. The WS locus (WRN) encodes a protein that shows significant sequence homology to the RecQ family of DNA helicases. Because a DNA helicase may function in DNA mismatch repair, we examined extracts of WS cell lines for mismatch repair activity. Extracts from four different WS lymphoblastoid cell lines containing different WRN mutations and from three within-pedigree control cell lines were all proficient in mismatch repair. In marked contrast, extracts from three independent WS fibroblastoid cell lines were deficient in repair of base-base and insertion/deletion mismatches. Extracts of one of these lines restored activity to extracts of mismatch-repair-deficient tumor cells with defined mutations in hMSH2, hMSH3, hMSH6, hMLH1, or hPMS2. This suggests that the WRN mutation in this fibroblast line is not a dominant negative inhibitor of mismatch repair activity and that the repair defect does not reside in these five known mismatch repair genes. Defective mismatch repair in fibroblastoid but not lymphoblastoid cells is consistent with the possibility that WRN protein could have a cell type- and/or tissue-specific role in mismatch repair. Alternatively, a mutation in WRN could predispose cells to mutations in other genes required for mismatch repair activity, at least one of which could be an unknown gene.

INTRODUCTION

WS (McKusick catalogue no. 277700) is an uncommon, autosomal recessive disease, the phenotype of which mimics premature aging. As young adults, WS patients develop bilateral cataracts, graying and loss of hair, osteoporosis, atherosclerosis, and diabetes. WS patients are also at increased risk of developing cancer. The most common malignant neoplasms are thyroid carcinoma, bone and soft tissue sarcomas, and malignant melanoma, and meningiomas and graying and loss of hair, osteoporosis, atherosclerosis, and diabetes. The increased risk of malignancy in WS patients could arise from a DNA repair defect. Precedents for such an association exist in the correlation between skin cancer and a defect in nucleotide excision repair in xeroderma pigmentosum patients (reviewed in Ref. 20). Defects in DNA mismatch repair are also linked to hereditary nonpolyposis colorectal cancer and to numerous sporadic cancers (reviewed in Refs. 21 and 22). Studies of the link between cancer and microsatellite instability have revealed the identities and functions of several of the genes and gene products involved in the early steps of DNA mismatch repair (reviewed in Refs. 22–24). Similar to the way Escherichia coli requires DNA helicase II for mismatch repair (reviewed in Ref. 25), a helicase could be required for mismatch repair in human cells. The identification of the WRN protein as a potential helicase and the causal links that have been established between loss of helicasenlike helicase-like proteins and genetic instability, elevated cancer risk, and DNA repair defects (26–28) prompted us to examine a possible role of the WRN protein in DNA mismatch repair in human cells.

MATERIALS AND METHODS

Cell Culture. The cell lines used in this study are described in Table 1. The WS SV40-transformed fibroblastoid cell line PSV811 and the WS and control B-lymphoblastoid cell lines were obtained from the International Registry for Werner Syndrome at the University of Washington (4). WS SV40-transformed fibroblastoid cell lines W-V and WS780/AG11395 were obtained from J. Carl Barrett (National Institute of Environmental Health Sciences). The WRN mutations in the B-lymphoblastoid cell lines and in WS780/AG11395 were determined by Oshima et al. (7). All other cell lines have been described previously (29, 30), except for the endometrial cell line HOUA, in which the hPMS2 protein is not detectable.

WS and control B-lymphoblastoid cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan UT). Other cell lines were grown in a 1:1 mixture of DMEM and Ham’s F-12 medium, supplemented with 10% fetal bovine serum. All growth media were supplemented with 30 mg/liter penicillin G, 50 mg/liter streptomycin, and 2.5 mg/liter Fungizone® (Life Technologies, Inc.). Cultures were maintained at 37°C in a humidified 5% CO2 incubator.

Mismatch Repair and Replication Activity Assays. Preparation of cell extracts, sources of materials, construction of substrates, and all details of the mismatch repair and SV40 replication activity assays have been described (31, 32). Mismatch repair was investigated using heteroduplex...
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Table 1 Cell lines used in this study

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mutant allele position</th>
<th>Predicted protein</th>
<th>Comments (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SY90575</td>
<td>1&amp;2: CAG→TAG STOP/3724</td>
<td>1164 aa</td>
<td>Homozygous mutant (8)</td>
</tr>
<tr>
<td>SY90576</td>
<td>None</td>
<td>Wild type</td>
<td>Unaffected sibling potential heterozygote</td>
</tr>
<tr>
<td>ZM90630</td>
<td>1&amp;2: G→C/3370-3464</td>
<td>1060 aa</td>
<td>Homozygous splice site mutation leads to skipped exon, frameshift, and stop (8)</td>
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<tr>
<td>ZM90633</td>
<td>None</td>
<td>Wild type</td>
<td>Unaffected sibling potential heterozygote</td>
</tr>
<tr>
<td>AUS40025</td>
<td>1: A deletion/1395</td>
<td>391 aa</td>
<td>AUS mutant allele 1 (7)</td>
</tr>
<tr>
<td></td>
<td>2: GA→GT splice site mutation</td>
<td>1157 aa</td>
<td>AUS mutant allele 2 (7); splice site mutation creates 113-bp deletion encompassing cDNA nts 3691-3803 (8)</td>
</tr>
<tr>
<td>AUS40010</td>
<td>1: A deletion/1395</td>
<td>391 aa</td>
<td>Carrying father has AUS mutant allele 1</td>
</tr>
<tr>
<td></td>
<td>2: None</td>
<td>Wild type</td>
<td>Wild and type allele (7)</td>
</tr>
<tr>
<td>DW90650</td>
<td>1&amp;2: CGA→TGA STOP/1336</td>
<td>368 aa</td>
<td>Homozygous mutant (7)</td>
</tr>
<tr>
<td>SV40-transformed fibroblastoid cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSV811</td>
<td>1&amp;2: not yet defined</td>
<td>Not yet defined</td>
<td>Derived from 29 yr-old female WS patient (37); exhibits chromosome instability and spontaneous deletion mutator phenotype at the HPRT locus (15, 38)</td>
</tr>
<tr>
<td></td>
<td>W-V</td>
<td>1: G deletion/3004</td>
<td>Altered protein; W-V mutant allele 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: Not yet defined</td>
<td>Not yet defined</td>
</tr>
<tr>
<td></td>
<td>W-V mutant allele 2; line is phenotypically similar to PSV811</td>
<td>Derived from 45 yr-old male WS patient (39)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WS780/AG11395</td>
<td>1&amp;2: CGA→TGA STOP/1336</td>
<td>Homozygous for same mutation as OW pedigree; derived from 60 yr-old male WS patient (40); displays normal sensitivity to UV and DNA-damaging drugs (40)</td>
</tr>
</tbody>
</table>

**aa**: amino acid; nt, nucleotide.

RESULTS

Mismatch Repair in Extracts of WS B-Lymphoblastoid Cell Lines.

The WS cell lines used in this study are listed in Table 1, and the organization of the predicted WRN protein and the location of the various WRN mutations are shown in Fig. 1. When extracts of the lymphoblastoid cell lines were examined for mismatch repair, both WS and unaffected pedigree-control lines repaired a G-G mismatch, with an efficiency similar to that observed with a HeLa cell extract.

Table 2 Complementation analysis with mismatch repair-defective cell extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>PSV811 WRN⁺</th>
<th>W-V WRN⁻</th>
<th>WS780 WRN⁻</th>
<th>HEC59⁺/hMSH2⁺</th>
<th>HCT15⁺/hMSH6⁺</th>
<th>HHUA⁻</th>
<th>HOUA⁻/PMS2⁻</th>
<th>HCT116⁺/hMLH1⁺/hMSH3⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSV811</td>
<td>7.4</td>
<td>0.2</td>
<td>60</td>
<td>54</td>
<td>75</td>
<td>39</td>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td>W-V</td>
<td>2</td>
<td>14</td>
<td>37</td>
<td>52</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>WS780</td>
<td>5</td>
<td>60</td>
<td>48</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

**% repair**: Determined as described in "Materials and Methods." The substrate contained a G-G mismatch at position 88 and a 5' nick at position +276.

**a** The repair values listed are the average of two experiments, except for the values for W-V or WS780 in combination with HHUA, which are single determinations. Repair efficiency values below 10% are in the range of experimental fluctuation in the assay and should not be considered as evidence that strand-specific repair is occurring.

**b** The endometrial carcinoma cell line HEC59 is heterozygous for two mutations in hMSH2, one a CAA→TAA mutation in exon 7 and the other a 4-bases insertion in exon 9 (30).

**c** The colorectal carcinoma cell line HCT15 contains two hMSH6 mutations, a 1-bp deletion at codon 22 (Leu→non-sense) and a GATAGA→T mutation at codon 1103, creating a termination codon 9 bp downstream (41).

**d** The endometrial carcinoma HHUA cell line is homozygous for two mutations, one a deletion of an A-T base pair at position 1148 in the hMSH6 gene that results in a protein 723 amino acids shorter than the wild type and the other a homozygous C→T mutation at nucleotide 3655 of the hMSH6 gene, changing codon 1219 from conserved threonine to an isoleucine (42).

**e** The hPMS2 protein is not detectable by western blot in the HOUA endometrial carcinoma cell line.5

**f** The colorectal carcinoma cell line HCT116 is hemizygous in hMLH1 for a TCA→TAA truncation at codon 252 in exon 9 (43) and is also homozygous for the same 1-bp deletion in hMSH5 that is present in HHUA cells.5

M13mp2 DNA containing a single base-base mismatch or two extra bases in one strand. For example, one substrate contains a G-G mismatch and consists of a covalently closed (+) strand that encodes a colorless or "white" plaque phenotype and a nicked complementary (−) strand that encodes a blue plaque phenotype. The nick directs repair to the (−) strand and is located either 3' (G/G-A) or 5' (G/G-B) to the mismatch, which is located at position 88 of the lacZα-complementation coding sequence. When introduced into an E. coli strain deficient in methyl-directed heteroduplex repair (e.g., mutS), an un repaired G-G heteroduplex yields a "mixed" plaque phenotype due to expression of both strands of the M13mp2 DNA. However, if repair of the mismatched base situated on the (−) strand takes place during incubation with a human cell extract, the percentage of mixed plaques decreases and the percentage of colorless (−) plaques increases, such that the ratio of pure blue to pure colorless plaques is reduced. Detection of mismatch repair activity with this assay only requires excision of the mismatched base(s) in the nicked strand. Although DNA repair synthesis in the extract does occur under normal circumstances (33, 34), synthesis is not required because the gap generated by mismatch excision can be filled in by an E. coli polymerase after introduction of the DNA into the α-complementation host [see Fig. 1 in Umar et al. (35)]. The calculation of mismatch repair efficiency is described in Thomas et al. (31). Details of individual experiments are given in the legends to Table 2 and Figs. 1–3.
Repair was proficient, with substrates containing the nick either 3' or 5' to the mismatch (Fig. 2A). That repair was strand specific is indicated by the decrease in the ratio of blue to white plaques (Fig. 2A, inset). Proficient repair of substrates containing a 2-base loop was also observed in all of the WS-afflicted and unaffected pedigree-control cell lines. Again, repair was strand specific (Fig. 2B, inset) and was observed when the nick was either 3' or 5' to the loop (Fig. 2B).

**Mismatch Repair in Extracts of WS Fibroblast Cell Lines.** We then examined mismatch repair in extracts of the WS fibroblastoid cell lines. In these experiments, a (positive control) HeLa cell extract of substrates containing the G-G mismatch and either a 5' or a 3' nick (Fig. 3), and the blue to white plaque ratio declined, as expected for stand-specific repair (Fig. 3, inset). However, extracts of the WS cell lines PSV811, W-V, and WS780 were all defective in repair of these substrates (Fig. 3). The repair deficiency in the WS780 cell extract is particularly interesting because WS780 cells contain the same WRN mutation present in the OW lymphoblastoid cell line that was repair proficient (Fig. 2).

Extracts of PSV811 were also found to be defective in repair of a substrate containing a T-G mismatch or one, two, or four unpaired bases (data not shown). Each of the three WS fibroblast extracts was competent for SV40 origin-dependent DNA replication (see legend to Fig. 3), demonstrating that they are not generally deficient in all DNA transactions. Moreover, no inhibition of repair of a G-G mismatch by repair-proficient HeLa cell or WS lymphoblastoid cell extracts was observed when these were mixed with PSV811, W-V, or WS780 extracts (data not shown). Thus, extracts of WS fibroblastoid lines did not contain a trans-acting inhibitor of repair. Extracts of two cell lines obtained by SV40 transformation of normal fibroblastoid cells, ID4 (36) and GM637 (15), efficiently repaired these same substrates, demonstrating that SV40 transformation alone does not inhibit mismatch repair activity in fibroblastoid cells. (The extract of GM637 cells was prepared using a protocol that was slightly modified from the standard methods of extract preparation described in Ref. 31.) These results do not rule out the possibility that SV40 transformation in conjunction with the presence of WRN mutations confers a mismatch repair deficit in fibroblasts.

**Complementation of Repair-deficient Extracts.** In an attempt to determine the defects responsible for the mismatch repair deficiencies observed in extracts of the WS fibroblast cell lines, we performed G-G mismatch repair reactions using pairwise combinations of mismatch repair-defective extracts. First, extracts of PSV811, W-V, and WS780 cells were used in pairwise combinations with each other. Efficient repair was observed when PSV811 and WS780 were included in the same reaction, but little or no repair was observed with the other two combinations (Table 2). This suggests that the nature of the mismatch repair defects is different in these WS fibroblast cell lines. This same suggestion derived from studies in which extracts of PSV811, W-V, and WS780 cells were used in pairwise combinations with extracts of mismatch repair-deficient human tumor cell lines harboring defined mutations in five different known mismatch repair genes (Table 2).

The addition of a PSV811 extract restored repair to reactions containing mismatch repair-defective extracts prepared from cells mutant in hMSH2 (HEC59), hMSH6 (HHUA and HCT15), hMSH3 (HHUA and HCT116), hPM2 (HOUA), or hMLH1 (HCT116; Table 2). In contrast, addition of an extract of W-V cells restored repair to reactions containing mismatch repair-defective extracts prepared from cells mutant in hMSH2 (HEC59), hMSH6 (HCT15), or hMSH3 and hMLH1 (HCT116), but not to extracts of HOUA cells (lacking hPM2 protein) or extracts of HHUA cells mutant in hMSH3 and hMSH6 (Table 2). The results with an extract of WS780 cells were similar to those obtained with an extract of W-V cells, except that complementation of an extract of HCT116 cells was minimal (11%; Table 2). In all cases where functional complementation of repair was observed, the change in the ratio of blue to white plaques relative to the unrepaird control was consistent with strand-specific repair.

**DISCUSSION**

This study was undertaken to determine whether the WS gene product functions in mismatch repair. Extracts of four different EBV-transformed lymphoblastoid cell lines containing characterized mutations in the WRN gene (Table 1 and Fig. 1) were all proficient for repair of a G-G mismatch (Fig. 2A). This mismatch is a good substrate for the general mismatch repair system in humans that requires both the MutSa heterodimer of hMSH2 and hMSH6 and the MutLa heterodimer of hMLH1 and hPM2 (for review see Refs. 23 and 24). The WRN-mutant lymphoblastoid cell extracts were also proficient in the repair of a substrate containing two unpaired bases (Fig. 2B), a process thought to be initiated by binding of a heterodimer of hMSH2 and hMSH3. Proficient repair of both base-base and insertion/deletion substrates occurred despite the fact that the four WRN-mutant cell lines contain very different WRN mutations that, in at least one case (OW), eliminated potential helicase activity of the WRN protein (Fig. 1). OW is homozygous for a nonsense codon in the NH2 terminus of WRN, and the predicted protein consists of only 368 amino acids (Table 1). These results indicate that neither the complete WRN gene product nor predicted helicase activity encoded by the conserved helicase motifs plays an essential role in hMSH2/hMSH6- or hMSH2/hMSH3-dependent strand-specific mismatch repair in human B-lymphoblastoid cell line extracts. Thus, WRN may play no role in mismatch repair or that its functional may be redundant in B-lymphoblastoid cells.

In contrast to the results obtained with extracts of the lymphoid cell lines, extracts of all three WRN-mutant fibroblastoid cell lines were deficient in repair of a G-G mismatch (Fig. 3). None of the fibroblast extracts suppressed the mismatch repair activity of repair-proficient extracts, indicating that the repair deficiency of the fibroblast extracts is not due to a trans-acting inhibitor of repair. The observations that PSV811 is complemented by WS780 but not by W-V and that complementation of WS780 by W-V is minimal (Table 2; 14% repair, a borderline value) suggest that these three fibroblast lines harbor different mismatch repair defects. This possibility is supported by the dissimilar abilities of these fibroblast lines to complement extracts of tumor cell lines with mutations in known mismatch repair genes.
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Fig. 2. Mismatch repair in extracts of WS lymphoid cell lines. Reactions were performed and analyzed as described in Materials and Methods. The efficiency of repair is calculated from the reduction in mixed bursts relative to an unrepaired mock reaction (containing distilled water rather than extract) and expressed as a percentage. Cell lines derived from WS-afflicted patients are italicized and unaffected pedigree controls are not (see Table 1). A, repair efficiency of G-G-A and G-G-B mismatches. The substrate containing the G-G mismatch at position 89 is designated "A" when the nick is 3' to the mismatch at the AvaII site (position -264) or "B" when the nick is 5' to the mismatch at the Bsu36 I site (position +276). (Position 1 is the first transcribed base of the lacZ a-complementation sequence.) In both cases, the nick is situated in the (−) strand. Inset, ratio of blue to white plaques. B, repair efficiency for substrate containing two extra bases (112) in the plus strand, designated (+) or in the (−) strand, designated (−). "A" and "B" refer to the orientation of the nick, as described above. Repair of Δ2 + A results in a blue plaque phenotype, whereas repair of Δ2 − B yields a white plaque phenotype. Hence, repair changes the blue:white ratio in opposite directions, as shown in the insert.

Fig. 3. Mismatch repair in extracts of WS fibroblast cell lines. Repair efficiency of G-G-A and G-G-B mismatches. Reactions were performed and analyzed as described in the legend to Fig. 1. Inset, blue:white ratios of the G-G reactions. All extracts were competent for SV40 origin-dependent replication, with incorporation values exceeding by at least 10-fold those obtained in control reactions containing extract but no T antigen. The HeLa cell extract (50 μg) incorporated 278 pmol of total nucleotide in a 2-h reaction; the comparative replicative capacities of WS cell extracts, expressed as a percentage of the HeLa extract, were: W-V, 15%; WS780, 13%; and PSV811, 75%.

(Table 2). The data are consistent with the possibility that W-V may be hPMS2-deficient and WS780 may be deficient in hPMS2, hMLH1, or hMSH3. Further work is required to resolve these possibilities.

The most intriguing results in the present study were obtained with extracts from PSV811 cells. The PSV811 cell line exhibits chromosomal instability and has an elevated HPRT mutation rate and a deletion mutator phenotype, properties that may reflect the deficiency in mismatch repair activity in extracts observed here (Fig. 3). However, PSV811 does not display detectable microsatellite instability at any of five loci that have thus far been examined (37). Cumulatively, the properties of PSV811 cells are distinct from those observed in cells harboring mutations in hMSH2, hMSH3, hMSH6, hMLH1, or hPMS2. One possible explanation for these differences is that PSV811 cells may harbor a mutation in an unknown mismatch repair gene. This is consistent with the observation that an extract of PSV811 cells is not demonstrably deficient in any of five known mismatch repair proteins (Table 2). Alternatively, it is possible that PSV811 cells retain a bevel of mismatch repair activity that is too low to be scored above the noise of the extract activity assay used here (~10%, see ref. 31).

It is remarkable that three of three WS fibroblastoid cell lines examined were repair deficient, whereas four of four WS lymphoid

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cell lines were repair proficient. These results are consistent with at least two possibilities. One is that the WRN gene does participate in mismatch repair, but its function is redundant in B-lymphoblastoid but not fibroblastoid cells. Another is that a mutation in the WRN gene may predispose some but not all cell types to secondary mutations in mismatch repair genes that lead to loss of mismatch repair activity. An elevated mutation rate in WS cells, as exemplified by the elevated HPRT mutation rate and the deletion mutator phenotype of the PSV811 cell line (15), could give rise to such secondary mutations. This possibility is intriguing given the strong link between loss of mismatch repair activity and tissue-specific tumorigenesis and the restricted spectrum of neoplasms observed in WS patients (2). It should be possible to test whether a cell lineage-specific expression of functional deficits occurring in conjunction with mutations at the WRN locus contributes to the unusual tumor spectrum and the different types of cutaneous and soft tissue pathology displayed by WS patients.

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REFERENCES


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